

=> d his

(FILE 'HOME' ENTERED AT 11:12:08 ON 01 APR 2002)

FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 11:12:24 ON 01 APR 2002

L1	342 S SYBR (W) GREEN AND (PCR OR POLYMERASE(W) CHAIN OR MELTING (W)
L2	60 S L1 AND FY<1998
L3	30 DUP REM L2 (30 DUPLICATES REMOVED)

=>

DOCUMENT-IDENTIFIER: US 6323337 B1

TITLE: Quenching oligonucleotides

----- KWIC -----

DEPR:

Oligonucleotide primers labeled with quencher dyes, prepared as described above (Example 8), are separated by electrophoresis in polyacrylamide or agarose gels, or are separated in capillary electrophoresis, or using microfluidic methods, along with unlabeled primers and PCR products or other products of primer extension. The gels are then stained with an appropriate nucleic acid gel stain, such as ethidium bromide, SYBR Green I stain, SYBR Green II stain, a red fluorescent SYTO stain, SYBR Gold stain, SYTO blue stain, SYTO green stain, or SYTO orange stain. Alternatively, the capillary electrophoresis is performed in the presence of the stain, using standard methods. Primers labeled with the quencher dyes are essentially nonfluorescent in the presence of the fluorescent nucleic acid stain, and thus do not contribute appreciably to the staining pattern in the gel. This simplified pattern facilitates automated gel or capillary electrophoresis analysis. Similarly quenched primers or ligation monomers can be eliminated from the staining pattern in a ligation assay containing one labeled and one unlabeled oligonucleotide, or a telomerase assay. Primer dimers with quenchers on both 5' ends are also not detected by fluorescence because their fluorescence is essentially fully quenched, so that even if they are abundant they do not obscure signals due to short amplification products.

DEPR:

PCR reactions are prepared, using oligonucleotide primers labeled with quencher Compound 16 or 19. SYBR Green I stain is included at a dilution of 1:50,000 of the commercially available stock solution, or PICOGREEN reagent is added to the reaction after PCR is completed at a final concentration of 0.8 .mu.M, and the fluorescence of the solution is measured. If SYBR Green I stain is included in the reaction, then the reaction can be monitored in real time, using an appropriate instrument, such as the LIGHTCYCLER (Roche) or the GENEAMP 9700 (Perkin Elmer). The background fluorescence in reactions containing quenched primers is lower than that observed in those containing unlabeled primers, and in addition, primer dimers do not contribute to the product signal. Other stains, such as YOYO-1 or OLIGREEN reagent, are added to the solution after PCR with the same results. Other stains, such as YO-PRO-1, are added to the solution prior to or during PCR with essentially the same results.

DETL:

TABLE 3 Fluorescence quenching by selected quenched oligonucleotides when associated with selected nucleic acid stains. Relative Fluorescence.sup.1

Quenching Moiety	Nucleic acid	Ex/Em.sup.2	Free stain (nm)
dye.sup.3	DABCYL		
Cpd. 6	Cpd. 8	Cpd. 11	Cpd. 14
Cpd. 16	Cpd. 19	POPO-1	
434/456	2.6	8.7	10
6.1	3.5	6.1	3.5
PO-PRO-1	435/455	9.9	23
21	25	21	22
SYTO	43		
438/460	35	39	35
43	41	44	
BOBO-1	462/481	16	30
40	33	41	27
SYBR Green I	494/521	3.7	
7.9	4.9	5.1	4.0
6.0			
2.7	28	PicoGreen reagent	493/525
3.2	9.4	5.3	4.2
3.6	6.8		
4.0	29	OliGreen	
reagent	498/515	4.3	9.6
7.1	5.2	4.3	6.4
4.2	27	SYBR Gold	
stain	494/530	3.2	
7.7	4.0	4.0	4.0
5.3	YO-PRO-1	491/509	4.3
16	9.9	8.8	6.5
8.9	6.0	34	YOYO-1
491/509	13	24	23
11	12	9.2	8.9
9.2	Ethidium bromide		
518/605	95	99	87
97	86	89	

75 83 JOJO-1 529/545 1.7 2.8 1.4 BOBO-3 570/604 76 60 56
 64 60 64 YOYO-3
 612/631 6.9 38 28 8.9 5.2 5.1 SYTO 59 630/645 9.1 61 27 15
 6.3 11 SYTO 61
 630/645 12 60 37 23 6.9 12 .sup.1 Relative fluorescence is
 the percentage of
 fluorescence exhibited by an quenching moiety-labeled
 oligonucleotide stained
 with the indicated nucleic acid stain relative to that of
 an unlabeled
 oligonucleotide of the same sequence, stained with the
 same amount of nucleic
 acid stain. .sup.2 Ex/em designates the fluorescence
 excitation and emission
 maxima for the indicated nucleic acid stain, bound to
 double-stranded DNA.
 .sup.3 Free dye indicates the relative fluorescence
 exhibited by the nucleic
 acid stain alone, expressed as a percentage of the
 fluorescence of the
 unlabeled oligonucleotide of the same sequence, bound to
 the same amount of
 nucleic acid stain. In some cases, the binding of
 particular quencher
 conjugates reduced the fluorescence of the nucleic acid
 stain solution to a
 level lower than that observed for free, unbound stain.

DOCUMENT-IDENTIFIER: US 6316198 B1

TITLE: Detection of mutations in genes by specific LNA primers

----- KWIC -----

DRPR:

FIG. 6. Melting curve analysis of PCR fragments generated with the EQ3053/EQ3213 primer set and either the "A-allele" template (A, lower curve) or the "G-allele" template (G, upper curve). The upper panel shows the fluorescence of the SyBR Green I dye bound to double-stranded amplicon. The lower panel shows the first negative derivative ($-dF/dT$) of the melting curve in upper panel. The T.sub.m is seen as the peak on the $-dF/dT$ plotting.

L Number	Hits	Search Text	DB	Time stamp
1	130	sybr near3 green	USPAT; US-PGPUB	2002/04/01 11:04
2	36	(sybr near3 green) same (allele\$ or polymorphism or oligonucleotide)	USPAT; US-PGPUB	2002/04/01 11:05